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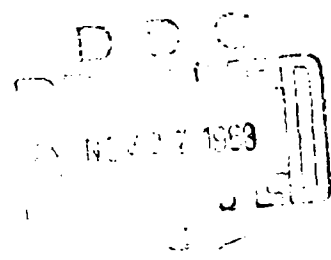
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DEPARTMENT OF THE ARMY
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Frederick, Maryland

EHRLICH'S DIAZO REACTION

Z. Klin. Med. (J. Clin. Med.)
10: 561, 573, 1894

F. Brewing

The ability of the diazo compounds of producing a variety of color combinations in combination with a large number of aromatic substances induced Ehrlich to engage in research on the reaction of human urine to such diazotized compounds in order to extend the diagnostic aid furnished by the urine. He utilized for this a mixture containing sulfanilic acid, hydrochloric acid, small amounts of nitrite and small quantities of diazobenzene sulfonic acid. This reagent is mixed with the urine to be tested and then supersaturated with ammonia. Whereas the greater number of the specimens then shows only a somewhat more distinct yellow coloration, a number of the specimens from patients acquire a red color varying over a scale from bright pink to deep red. From the presence or absence of the diazo reaction as well as the character of the sediment showing a greenish tone under certain circumstances, Ehrlich drew certain diagnostic conclusions which he embodied in the following statements:

- 1) The reaction is one of the most constant signs of typhoid fever and its absence makes a diagnosis of the former doubtful.
- 2) If a patient with an initial diagnosis of apparent typhoid shows a very minor or no reaction at all between five and eight days later, then the case can be regarded a priori as extremely light with a consequent prognosis.
- 3) Catarrhal gastritis (status gastricus) never shows a reaction.
- 4) If the reaction declines or disappears while the fever remains constant, a remission can be expected in general within a few days.

5) An intense and prolonged reaction may occur also with slight forms of the disease and therefore does not justify, especially for female patients, an unfavorable prognosis.

6) Relapse typhoid customarily shows an excessive reaction and the latter therefore need not lead to an unfavorable prognosis.

7) If, during a case of typhoid, there occurs an increase of fever which is prolonged and accompanied by a strong reaction, then this is an exacerbation of the typhoid process and a consequent protracted course of the illness is probable.

8) Severe prostration of the bodily forces such as occurs, during a severe case of typhus, with or without the intercession of a localized illness, e.g. hypostasis, lobar pneumonia, may cause disappearance of an already existing strong reaction. If this reaction disappears at the height of the typhus together with a manifest deterioration of general health, this may be regarded as a turn for the worse.

9) The same reaction is found in epidemic typhus and military tuberculosis and its presence therefore is no aid in differential diagnosis of these "three" diseases.

The conclusions of Ehrlich as well as the dissertations of Fischer, Brecht and Lowinson prepared under the supervision of Ehrlich were soon strongly contradicted by Pentzold and Petri who alleged observation of this reaction also in normal urine and consequently disputed the diagnostic value of this reaction. The differing findings of Pentzold and Petri were due, according to Ehrlich, to the use of excessively strong solutions of the diazo compounds whereas Ehrlich found that the success of his reaction is dependent on the presence of small amounts of the active diazobenzene sulphonic acid. The conclusions of Ehrlich were soon confirmed by Escherich but a reexamination by Pentzold based on sixty-nine individuals with two hundred and sixty-five individual specimens allegedly made strictly within the sense of the instructions by Ehrlich did not revise the opinion of this author on the diagnostic utility of the reaction. Pentzold embodied this conviction in the following statements:

1) The substances conditioning the reaction are of different kinds and are not yet known to us.

2) The change of color observed in many diseases where the reaction has been used, are not actually qualitative but represent all possible transitions from yellow to red and exhibit almost constantly the admixture of the yellow tone of the urinary pigments.

3) Although the reaction does occur in certain diseases almost regularly and in others less frequently, it can occur in the greater majority of feverish and a part of the non-feverish illnesses so that it has only little use for their differentiation.

According to Spiethoff who upholds the views of Ehrlich as against Pentzold, a green sediment is always precipitated if the true reaction exists and only this sediment is useful in diagnosis. In a further polemic article, Petri again attacked Ehrlich on the basis of two hundred cases of pulmonary tuberculosis in which the reaction largely failed in regard to its useful prognosis and diagnosis. Brehmer also was not able to find that the intensity of the diazo reaction parallels the intensity of the fever. The occurrence of the reaction indicates in his observations not always a deterioration of the condition whereas Grundies regards the occurrence of the reaction in pulmonary tuberculosis always as an ominous sign.

With this dispute among the various authors, a reexamination was rather necessary and especially so since there was a risk of oblivion for the reaction due to the derogatory judgement of various authors.

Although it had become evident that the original conclusions of Ehrlich had undergone certain restrictions, the reaction did offer certain features of clinical interest. Obviously, an evaluation of the significance of the reaction could be obtained only on the basis of a larger body of material which was amply available to me at the former Clinic of v. Frerich and that I examined in this regard at the wish and under the direction of Professor Brieger.

However, before I enter on the observations made in the clinic, I should like to stress that it is not necessary for the evaluation of a reaction, as demanded by Pentzold, to accurately know the substance which is the basis of the reaction. I shall recall here only the red color of urine with iron chloride in diabetes and other diseases leading to cachexia, a reaction which always plays a certain role although its indicator has not yet been definitely confirmed.

The observations here reported cover a period of six months. The urine of 265 patients was examined once per day, with the reagent prepared according to the instructions of Ehrlich, as long as the patients remained in the clinic. The number of individual tests was about 2,500. Among the 265 patients observed, a diazo reaction was shown in 47 whereas the reaction was absent among the other 218 patients during

their entire stay in the clinic. The 47 cases of diazo reaction observed were distributed among the following illnesses:

Childbed fever	11
Lobar pneumonia	8
Typhoid fever	7
Pulmonary tuberculosis	6
Facial erysipelas	4
Scarlet fever	3
Measles	2
Leukemia	2
Intestinal tuberculosis	1
Purulent flux; Parametritis, 4th month of pregnancy	1
Metrorrhagia, 4th month of pregnancy	1
Multiple suppurative hepatitis and purulent portal periphlebitis	1
Total	47

The 218 patients in which the reaction was absent during their entire stay in the clinic suffered from the following illnesses:

Follicular tonsillitis	19
Rheumatic polyarthritis	10
Vitium cordis	18
Anemia	18
Purulent flux	13
Gastroenteritis	11
Stomach ulcer	10
Nephritis	9
Lobar pneumonia	9
Catarrhal bronchitis	8
Catarrhal jaundice	7
Faucial diphtheria	6
Stomach cancer	5
Sacculated pleurisy [<u>"Pleuritis sicca"</u>]	5
Facial erysipelas	4
Cirrhosis of the liver	4
Scarlet fever	3
Muscular rheumatism	3
Epilepsy	3
Oxalic-acid intoxication	3
Senile marasmus	3
Pulmonary tuberculosis	2
Measles	2
Transverse myelitis	2

Chorea	2
Rheumatoid arthritis	2
Floating kidney	2
Suppurative pleurisy	2
Gastralgia	2
Abortus	2
Vomiting of pregnancy	2
Tuberoular meningitis	1
Purulent arachnitis	1
Occupational neurosis	1
Right hemiplegia and apoplectic hemorrhagy	1
Paralysis agitans	1
Paralytic dementia	1
Coccygodynia	1
Cardialgia	1
Carcinomatous peritonitis	1
Typhlitis /appendicitis/	1
Cholelithiasis	1
Epistaxis	1
Bronchiectasis	1
Chickenpox	1
Perimetritis	1
Sulfuric-acid intoxication	1
Carbon-monoxide intoxication	1
Mercurial stomatitis	1

Total 218

The illnesses in which the diazo reaction was observed can be grouped as follows:

1. Those illnesses in which the diazo reaction always existed for a certain interval of time: (1) childbed fever, 11 cases; (2) typhoid fever, 7 cases; (3) leukemia, 2 cases.

2. Those illnesses in which the diazo reaction was observed only in some patients whereas it was absent in other patients suffering from the same illness:

<u>Illness</u>	<u>Diazo Reaction</u>	
	<u>Yes</u>	<u>No</u>
Lobar pneumonia	8	9
Pulmonary tuberculosis	6	2
Facial erysipelas	4	4
Scarlet fever	3	3
Measles	2	2
Purulent flux	1	13

The duration in time of the diazo reaction as well as its relation to body temperature in the individual illnesses are indicated from the following observations based on the scale of intensity of Ehrlich: R_3 = very strong; R_2 = strong; R_1 = weak reaction.

I. Childbed Fever

Case 1: age 21; 13 days after delivery;

26.	Nov. Morning	39.5° C.	Evening	38.6° C.	R_1
27.		40.7		38.7	R_1
28.		38.3		38.8	R_1
29.		39.1		39.5	0
30.		38.3		39.3	0

Gradual regression of fever; no diazo reaction while in clinic.

Case 2: age 21; 25 days after abortus, pneumonia of right lower lobe

4.	Dec. Morning	38.3° C.	Evening	38.3° C.	R_1
5.		39.1		37.4	R_1
6.		39.2		39.0	R_1
7.		40.2		39.4	R_1
8.		40.0		39.1	R_1
9.		39.9		37.0	R_1
10.		38.6		36.2	0
11.		35		36.0	0
12.		36.0		36.0	0
13.		37		38.4	0
14.		37.9		37.2	0
15.		38.0		39.1	0
16.	Exitus letalis.				

Autopsy findings: status puerperalis (5 weeks after abortus); ulcerating endometritis; thrombophlebitic metritis; circumscribed right pulmonary gangrene; bilateral metastatic pneumonia; double metastatic pleurisy; pulmonary and glottic edema; myocardial adipose metamorphosis; pulpy hyperplasia of the spleen; parenchymatous nephritis and hepatitis.

Case 3: age?; 13 days after delivery; strongly delirious upon admission; much albumin in urine; pneumonia of left lower lobe

28. Jan.	morning	37.7° C.	Evening	39.7° C.	
29.	.	38.2	.	39.0	R ₁
30.	.	37.6	.	39.0	0
31.	.	38.3	.	37.7	0
1. Febr.	.	38.1	.	38.9	0
2.	.	38.3	.	39.2	0
3.	.	38.6	.	40.1	0
4.	.	39.0	.	39.3	0
5.	.	38.4	.	39.6	0
6.	.	38.8	.	39.7	R ₁
7.	.	40.4	.	37.0	R ₁
8.	.	37.9	.	40.0	R ₁
9.	.	38.8	.	38.7	R ₁
10. Febr.	.	39.8° C.	.	39.7° C.	R ₁
11.	.	38.3	.	40.5	R ₁
12.	.	38.8	.	38.8	0
13.	.	40.3	.	39.0	R ₁
14.	.	39.5	.	38.5	R ₁
15.	.	37.7	.	37.6	0
16.	.	39.3	.	38.0	0
17.	.	37.9	.	38.4	0
18.	.	39.0	.	40.1	R ₁

continuous fever and reaction of varying intensity; exitus on 1 March.

Autopsy findings: status puerperalis; right purulent-fibrinous pleurisy; right pulmonary infarct; hypertrophica cordis; parenchymatous myocarditis; hyperplasia of spleen; chronic parenchymatous nephritis; right pampiniform plexal thrombophlebitis.

Case 4: age 26; 12 days after delivery; left fibrinous pneumonia; right fibrinous pleurisy

2. März.	morning	40.0° C.	R ₁
3.	Evening	39.4° C.	40.3 R ₁
4.	.	39.9	40.2 R ₁
5.	.	37.6	39.0 R ₁
6.	.	39.0	38.6 R ₁

fever and reaction continue. exitus on 13 March.

Autopsy findings: status puerperalis; vaginal diphtheria; purulent metritis and endometritis; hepatitis; parenchymatous and left embolic nephritis; pulpy hyperplasia of spleen; double suppurative pleurisy; bilateral pleuropneumonia; chronic gastritis.

Case 5: age 25, ten days after delivery

14. Nov.	Evening	40.4° C.	R ₁
15.	Morning	37.6° C.	38.1 R ₁
16.	.	37.0	37.0 R ₁
17.	.	36.9	37.9 R ₁
18.	.	36.1	37.5 0

continuously without fever and without reaction.

Case 6: age 26; 13 days after delivery

8 Jan. Morning	37.6° C.	Evening	39.0° C.	R ₁
9	38.5 .		38.0 .	R ₁
10	37.0 .		37.0 .	0

continuously without fever and without reaction.

Case 7: age 28, 12 days after delivery. Temperature: evening of 10 March, 41.0° C. R₃; morning of 11 March, 39.6° C and R₃. exitus during evening.

Autopsy findings: puerperal sepsis; purulent pneumonia with hemorrhaging fibrinous metastases, and ichor form embolism of pulmonary artery; retention of placenta; diphtherial endometritis; thrombophlebitic metritis.

Case 8: age 43; 10 days after delivery

2. Mrs. M. Morning	37.6° C.	Evening	38.6° C.	R ₁
3.	37.0 .		38.5 .	R ₁
4.	37.7 .		37.7 .	R ₁
5. Mrs. Morning	38.1° C.	Evening	39.9° C.	0
6.	37.6 .		38.5 .	0
7.	37.3 .		37.4 .	0

continuously without fever and without reaction.

Case 9: age 28, 11 days after delivery. pneumonia of left lower lobe

2. Mrs. Morning	39.5° C.	Evening	40.0° C.	R ₁
3.	39.7 .		39.8 .	R ₁
4.	39.0 .		39.5 .	R ₁
5.	39.9 .		39.0 .	R ₁
6.	39.0 .		39.6 .	R ₁
7.				Exitus letalis.

Autopsy findings: status puerperalis; incomplete rupture of vagina and cervix; gangrenous diphtherial endometritis; chronic parenchymatous nephritis; pulmonary edema; sero-fibrinous hemorrhagic pericarditis; double pneumonia of lower lobe.

Case 10: age 29; 5 days after delivery

8. Jan. Morning	39.5° C.	Evening	39.2° C.	R ₁
9.	40.0 .		39.2 .	R ₁
10.	40.1 .		38.8 .	R ₁
11.	40.6 .		39.2 .	R ₁
12.				Exitus letalis.

Autopsy findings: status puerperalis; endometritis and hemorrhagic diphtherial colpitis; partial retention of placenta; phlegmonous thrombophlebitic metritis and perimetritis; pulpy hyperplasia of spleen; infarct of spleen; bilateral lower lobar metastatic pneumonia; multiple partial bacterial nephritis.

II. Typhoid Fever

On the duration of the diazo reaction in typhoid fever, we had available seven cases. The days with evening temperatures above 39° C are designated by bold print in the table below.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Age 31	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R
2. Age 21	-	-	-	R	R	R	R	R	R	R	R	R	-	-	-
3. Age 29	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R
4. Age 12	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R
5. Age 26	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R
6. Age 21	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R
7. Age 39	This patient also suffered from pulmonary tuberculosis														

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
R	R	O	O	O	O	O	O	O	O	O	O	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	O	O	O	O	-	-	-	-	-	-	-
R	O	O	O	O	-	-	-	-	-	-	-	-	-	-	-
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

key to table: 1. discharged as cured after 29 days;
2. exitus after 12 days; 3. discharged as cured after 47 days; 4. discharged as cured after 49 days;
5. discharged as cured after 32 days; 6. discharged

as cured after 39 days: 7. A strong diazo reaction was observed. Exitus after 41 days.

III. Leukemia

We observed two cases of leukemia, one a splenic form and one a pseudo-leukemia. Although the temperatures of both patients never appreciably varied and subnormal temperatures to 34° C were noted only in the last days of the pseudo-leukemia, the diazo reactions in both cases greatly varied in intensity every day. Whereas the reaction was entirely absent on individual days, a diazo reaction of R₃ repeatedly occurred already 24 hours later. Both cases terminated in exitus. Since the splenic leukemia was discharged from the hospital in a terminal state, we have available here only the autopsy findings of the pseudo-leukemia which showed:

Subject patient, age 33. Lymphosarcoma of the neck, of the posterior mediastinum, of the epigastric glands, of the retroperitoneum, of the lumbar region, of the groin, of the spinal column, of the liver, of the spleen. Amyloid degeneration of the spleen, the intestine and the kidney. Parenchymatous nephritis. Adipose degeneration of the renal cortex. Atrophy of the myocardium. Bilateral multiple lobar pneumonia. Ulcerating colitis. Typhous scars in ileum. Scars in vagina. Adhesive perimetritis.

IV. Lobar Pneumonia

We observed 14 cases of lobar pneumonia which took a typical course and of which only one was fatal at the height of the illness. These can be classified in three groups in regard to the reaction:

- 1) The reaction was absent in nine patients during the entire illness. Except for one fatal case, all others ended favorably.
- 2) The reaction was shown by two patients prior to the crisis of the illness. The regression of the pneumonic infiltrates took place rather rapidly and the patients recovered completely within one to two weeks.
- 3) The reaction occurred in three patients only after the crisis in the period of regressing fever. However, the resolution of the pneumonic infiltrates took a normal course in these cases.

In contrast to the lobar pneumonia, the "asthenic" form of pneumonia observed on three other cases always showed the reaction already at the time of hepatization. The disease here was protracted and fatal in all cases.

V. Pulmonary Tuberculosis

Six of eight patients showed the reaction. Four of the cases with reaction were in extremis and died. The two other cases with reaction concerned incipient tuberculosis so that the diazo reaction was only very slight in this initial stage.

VI. Facial Erysipelas

Four of eight patients with this illness showed the reaction with varying intensity. One female patient who had shown a reaction of R_3 in the initial stage of the illness and suffered a relapse eight days after discharge from the hospital, at that time no longer showed the reaction.

VII. Scarlet Fever

Three of six cases of the illness showed the diazo reaction. Whenever it occurred, the highest intensity was present with the start of eruption of the exanthema and regularly disappeared with the beginning of desquamation.

VIII. Measles

Two of four cases exhibited the reaction which was each time of shorter duration in this illness than for scarlet fever and regularly disappeared with the crisis of the exanthema.

IX. Suppurative Hepatitis

A female patient, age 26, had been suffering allegedly of gastritis for one week which became rapidly complicated by an intensive yellow coloration and by constipation. Upon admission, the emaciated patient suffered, in addition to perceptible icteric color of the skin, of the conjunctiva, of the urine, and pale stools, spontaneous pain and sensitivity to pressure in the right hypochondrium but without any enlargement of the liver. Fever and diazo reaction were absent. The syndrome consequently corresponded to that of a catarrhal gastroenteritis which had resulted in a catarrhal jaundice. Twenty-one days after admission and at a time when the jaundiced appearance had begun to regress, there occurred a sudden onset of purulent parotitis accompanied by chills and temperatures to 38.5° C. With the onset of this, the reagent produced an intensive coloration of

the urine (always R₃ from now on). Since the purulent parotitis required surgical intervention, the patient was transferred to another station but died in spite of the operation.

Autopsy findings: ulcerating diphtherial colitis; suppurative portal thrombophlebitis; parenchymatous hepatitis and purulent portal periphlebitis; multiple suppuration; dilation and obstruction of the bile duct; universal jaundice; indurated hyperplasia of the spleen; hemorrhagic parenchymatous nephritis; parotitis; partial ulcerating diphtherial proctitis.

If we now summarize these detailed indications and compare them with the experiences of other authors, we can then state the following on the value of the diazo reaction:

- 1) The diazo reaction represents an indirect symptom which, similar to the splenic tumor and the fever, is of diagnostic value not in itself but only in consideration of the other symptoms.
- 2) The occurrence of the reaction is based on the fact that the substances created by the decomposition of the parenchyma of the body and/or of the pus, are resorbed and are consequently eliminated through the kidneys.
- 3) The reaction is of value for diagnosis and prognosis specifically in four illnesses:
 - (a) The reaction is present almost without exception in severe cases of typhoid fever, but is unusually absent in lighter cases. Accordingly, the existence of the diazo reaction should facilitate diagnosis of typhoid fever in a certain respect. However, we have no experience on the behavior of the reaction in the first phase of the illness. During a case of typhus, the reaction may gradually become less and this may indicate a favorable prognosis. From the point of view of differential diagnosis, it is of significance that cerebrospinal meningitis does not exhibit the reaction. By contrast, the reaction is usually very intense in miliary tuberculosis so that its existence is of no value for differentiation between miliary tuberculosis and typhoid fever.
 - (b) In pulmonary tuberculosis, the reaction is present in advanced cases as was shown by Grundies and Germain-Sée although their findings do not agree with our observations which are, however, not very numerous. The reactions here indicates that a resorption of decomposed matter from the lungs takes place. However, in order for resorption to occur, both healthy and diseased tissue must maintain an active metabolism. This

is a condition which has satisfied for nonencapsulated caverns. Such a process directly indicates an unfavorable prognosis.

(c) The reaction occurs extraordinarily easily in puerperal affections) This is explained by the fact that the conditions for resorption are very favorable on the inner surface of the uterus. The reaction occurs frequently simultaneously with the fever but may also appear in advance of the latter. Accordingly, its symptomatic significance can therefore probably be equated with that of fever. It would therefore be desirable to pay very strict attention to the diazo reaction during the period of lying-in in order to provide very thorough intra-uterine disinfection as soon as the reaction is present. It should be stressed here that the prognosis of lying-in is by no means unfavorable a priori when the reaction occurs.

(d) The reaction can be very useful for the diagnosis of hidden suppuration, e.g. abscess of the liver, as shown by the case detailed above.

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